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Volatile Constituents and Antimicrobial Activities from Flower and Fruit of *Arbutus unedo* L.

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Essential oils isolated by hydrodistillation from flower and fruit of *Arbutus unedo* growing in Turkey were analyzed by GC and GC/MS. Forty-nine compounds, representing around 95 % of the total oils were identified. Non-terpenoids hydrocarbons were shown to be the main group of constituents of the flower and fruit part of the plant in the ratio of 62.2 and 92.8 %, respectively. The major component in the essential oil of the flower was α-terpineol (16.3 %) and the main compound in the essential oil of the fruit was hexadecanoic acid (21.7 %). The antimicrobial activity of the isolated essential oils of the flower and fruit were also investigated and it showed moderate antibacterial activity against *Listeria monocitogenes* and *Enterococcus faecalis*.

Key Words: *Arbutus unedo* L., Essential oil, Antimicrobial activity, GC-FID, GC-MS.

INTRODUCTION

The genus *Arbutus* L. (Ericaecea) represented with *A. unedo* L. and *A. andrachne* L. in Turkey[1]. *A. unedo* is widely distributed along the coast of Anatolia under the Mediterranean climates. It is mostly planted for ornamental usages, but its bright dark red fruit contain high amount of vitamin C and E[2,3]. The dried leaves is very rich of tannins, arbutins, thus it has been used as a tea in Anatolian folk medicine against to complaints of uterine cramps. The stem bark and dried leaf of *A. andrachne* is also used in Anatolia folk medicine[2].

Previous phytochemical studies on the aerial parts (leaf and fruit) of *A. unedo* has shown the isolation and identification of a number of compounds, such as terpenoids, anthocyanins, gallic acid derivatives, steroid, flavonoids, flavonol glycosides, iridoid glycosides, sugars, non-volatile acids, polyphenolic acids, carboxylic acids and derivatives and non-terpenoid C_6-C_{10} volatile organic compounds (VOC)[4-12]. The antioxidant and antimicrobial activities of the methanol, ethanol, water and hexane extracts and the essential oil of the leaf of *A. unedo* has been mentioned[3,13,14] and the amount of essential oil of the strawberry (*A. unedo*) fruit was also reported, but the

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constituents are not characterized[15]. Volatile of strawberry-tree honey (*A. unedo*), which was obtained by a dynamic headspace (DHS) extraction, has been analyzed by GC-MS and gave mainly aldehydes and ketones type compounds[8]. The literature search revealed no reports on the oil composition of the flower and fruit parts of the *A. unedo*. This paper reports the constituents of the essential oils of the flower and fruit of this species. The crude essential oils were investigated by GC-FID and GC-MS technique[16-26]. The identification of the substances was performed by comparison of retention indexes on HB-5 column (determined relatively to the retention times of a series of *n*-alkanes), authentic compounds and mass spectra with literature (Nist and Wiley)[16-26].

EXPERIMENTAL

A. unedo L. was collected in Yomra-Trabzon (at heights of *ca.* 440 m) in the northeastern part of Turkey in October 10, 2007. The plant was authenticated by Assoc. Prof. S. Terzioglu[1]. Voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO (KATO-4025), Karadeniz Technical University, Turkey.

Isolation of the essential oils: The fresh plant materials were separated into flower and fruit parts and they were freezed with liquid nitrogen and then grounded into small pieces. The essential oils from fresh aerial parts (*ca.* 110 g, each) of *A. unedo* were isolated by hydrodistillation in a Clevenger-type apparatus[27] with cooling bath (-15 ºC) system (4 h) (yields: 0.15 and 0.06 % (v/w), respectively). The obtained oils were extracted with HPLC grade *n*-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at 4-6 ºC in a sealed brown vial.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS): GC-FID and GC-MS analyses were described previously[28-30].

Identification of constituents: Retention indices of all the components were determined by Kovats method using *n*-alkanes (C_6-C_{32}) as standards. The constituents of the oils were identified by comparison of their mass spectra with those of mass spectral libraries (NIST and Wiley), authentic compounds (limonene, γ-terpinene, linalool, decane, pentadecane, heptadecane, nonadecane, heneicosane, tetracosane and pentacosane) and with data published in the literature[16-26].

Antimicrobial activity assessment: All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas auroginosa* ATCC 27853, *Listeria monocytogenes* ATCC 43251, *Bacillus cereus* 709 ROMA, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Candida tropicalis* ATCC 13803 and *Candida albicans* ATCC 60193. Essential oil constituents were weighed and dissolved in hexane to prepare extract stock solution of microgram/milliliter.

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration

(MIC) values (μg/mL) were determined[31]. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH.7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7, respectively. Mueller Hinton and Yeast Nitrogen Base broth medias containing 0.25 % (v/v) Tween 20 were used for the broth diffusion method. The MIC was defined as the lowest concentration that showed no growth. Ampicillin and fluconazole were used as standard antibacterial and antifungal drugs, respectively. Hexane with dilution of 1:10 was used as solvent control. The results are shown in Table-3.

RESULTS AND DISCUSSION

Altogether, 49 volatile compounds were identified by GC and GC-MS with HP-5 column from the essential oils of the flower and fruit parts of *A. unedo*[16-30]. The components of the oils, the percentage of each constituent and the retention indices are summarized in Table-1. The flower oil was revealed the presence of 35 volatile components, representing 95.3 % of the total oil; only 11 compounds of them were terpene and terpenoids. The major compounds of the flower oil were α-terpineol (16.3 %), hexadecanoic acid (14.9 %), nonanal (13.1 %), 2-pentylfuran (8.6 %) and (2E)-nonenal (5.3 %). The analyses of the fruit oil led to the identification of 21 constituents, accounting for 93.6 % of the total oil. The major constituents of the essential oil of the fruit were hexadecanoic acid (21.7 %), ethyl dodecanoate (13.4 %), ethyl linoleate (10.4 %), tetradecanoic acid (9.0 %) and ethyl linolenate (8.1 %). The number of volatile compounds present in the flower was greater than in fruit part of *A. unedo*. The results clearly indicate that the major constituents of the essential oil composition of the flower and fruit were different and only 12 components were the same in the ratio of 58.2 and 58.1 %, respectively. But, the fruit oil was rich in non-terpenoid components mostly aldehydes, carboxylic acids, esters and hydrocarbons (Table-2).

TABLE-1
IDENTIFIED COMPONENTS IN THE ESSENTIAL OILS OF *Arbutus unedo*[a,b]

Lit. RI/MS	Exp. RI	Compound	Flower	Fruit
			Area (%)	Area (%)
993	992	2-Pentylfuran	8.6	–
1000	1000	Decane[c]	2.3	–
1029	1029	Limonene[c]	1.4	–
1060	1061	γ-Terpinene[c]	0.4	–
1089	1089	α-Terpinolene	3.7	–
1097	1099	Linalool[c]	7.7	–
1101	1102	Nonanal	13.1	1.4
1114	1116	*trans*-Thujone	0.3	–
1162	1163	(2E)-Nonenal	5.3	1.6
1189	1192	α-Terpineol	16.3	0.6
1202	1204	Decanal	0.8	–
1264	1264	(2E)-Decenal	2.0	2.7

1271	1274	*trans*-Carane	0.9	6.4
1307	1308	Undecanal	1.1	–
1317	1318	(2E,4E)-Decadienal	3.0	1.7
MS1	1352	1,1,6-Trimethyl-1,2-dihydro-naphthalene	1.2	2.7
1409	1410	Dodecanal	0.5	–
MS2	1484	4-(2,6,6-Trimethylhexa-1,3-dienyl)-but-3-ene-2-one	0.9	–
1496	1497	2-Tridecanone	0.5	0.5
1500	1500	Pentadecane[c]	0.6	–
1506	1509	(E,E)-α-Farnesene	–	0.2
1595	1592	Ethyl dodecanoate	–	13.4
1613	1614	Tetradecanal	0.5	–
1640	1642	epi-α-Cadinol	0.4	–
1700	1700	Heptadecane[c]	0.9	–
1715	1715	Pentadecanal	0.5	–
1776	1777	Tetradecanoic acid	0.3	9.0
1847	1847	Hexahydrofarnesyl acetone	0.6	–
1900	1900	Nonadecane[c]	1.3	–
1919	1919	Farnesyl acetone	0.7	–
1922	1925	Methylhexadecanoate	0.4	0.6
MS3	1966	(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	0.7	–
1983	1983	Hexadecanoic acid	14.9	21.7
1993	1995	Ethyl hexadecanoate	0.4	3.6
2096	2095	Methyl linoleate	0.4	1.6
2100	2100	Heneicosane[c]	2.3	–
2101	2101	Methyl linolenate	–	1.2
2152	2154	Linoleic acid	–	1.2
2163	2163	Ethyl linoleate	0.4	10.4
2173	2171	Ethyl linolenate	–	8.1
2400	2400	Tetracosane[c]	–	0.9
2500	2500	Pentacosane[c]	–	4.1
		Total isolate	95.3	93.6
MS1	m/z (%)	150 (64), 135 (100), 121 (32), 107 (44), 93 (36), 79 (68)		
MS2	m/z (%)	190 (20), 175 (100), 147 (24), 105 (32), 91 (36), 55 (40)		
MS3	m/z (%)	272 (5), 145 (8), 132 (40), 119 (96), 93 (72), 68 (100), 55 (70)		

[a]RI calculated from retention times relative to that of *n*-alkanes (C_6-C_{32}) on HP-5 column; [b]Percentages obtained by FID peak-area normalization; [c]Identified with authentic samples.

The carboxylic acids and esters were the major constituents of the fruit of the plant in the ratio of 31.9 and 38.9 %, respectively. The major components in the oil of the flower were aldehydes (26.8 %) and monoterpenoids (25.2 %). In comparison with the previously reported composition of the essential oil of the leaf[3] with the flower and fruit of *A. unedo*, linalool, nonanal, (2E)-nonenal, α-terpineol, (2E)-decenal, (2E,4E)-decadienal, 2-tridecanone, hexahydrofarnesyl acetone and hexadecanoic acid were also detected in present oils samples with different ratios. It is necessary

to point out that the parts of the plant used strongly influence the chemical composition of essential oils as in present case.

TABLE-2
CHEMICAL CLASS DISTRIBUTION IN THE ESSENTIAL OILS OF *Arbutus unedo*

Compound class	Flower		Fruit	
	Area (%)	NC[a]	Area (%)	NC[a]
Monoterpenes	5.5	3	–	–
Monoterpenoids	25.2	4	0.6	1
Sesquiterpene	–	–	0.2	1
Sesquiterpenoids	1.7	3	–	–
Diterpene	0.7	1	–	–
Aldehydes	26.8	9	7.4	4
Carboxylic acids	15.2	2	31.9	3
Esters	1.6	4	38.9	7
Hydrocarbons	7.4	5	5.0	2
Others	11.2	4	9.6	3

[a]NC: Number of compounds.

The antimicrobial activities for the essential oils of the flower and fruit of *A. unedo* were tested *in vitro* using the agar-well diffusion method[31] with the microorganisms as seen in Table-3. The essential oil showed moderate antibacterial activity against *L. monocitogenes* and *E. faecalis*, but no activity was observed against the bacteria *E. coli*, *Y. pseudotuberculosis*, *P. auroginosa*, *B. cereus*, *S. aureus* and yeast like fungis *C. tropicalis* and *C. albicans*.

TABLE-3
SCREENING RESULTS FOR ANTIMICROBIAL ACTIVITY
OF THE ESSENTIAL OILS OF *Arbutus unedo*

Comp. No	Stok sol. (μg/100 μL)	Minimal inhibition concentration values (μg/100 μL)								
		Ec	Yp	Pa	Li	Bc	Sa	Ef	Ct	Ca
Flower	1500	–	–	–	750	–	–	750	–	–
Fruit	1500	–	–	–	750	–	–	–	–	–
Hexane	–	–	–	–	–	–	–	–	–	–
Ampicillin	10.000	8	32	32	2	> 128	2	< 1		
Flukonazol	50.000	–	–	–	–	–	–	–	8	25

Ec: *Escherichia coli* ATCC 25922, Yp: *Yersinia pseudotuberculosis* ATCC 911, Pa: *Pseudomonas aeruginosa* ATCC 10145, Bc: *Bacillus cereus* 702 Roma, Li: *Listeria monocitogenes* ATCC 43251, Sa: *Staphylococcus aureus* ATCC 25923, Ef: *Enterococcus faecalis* ATCC 29212, *Candida. tropicalis* ATCC 13803. (–): No activity of stok concentration.

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